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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

OCT 26 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: SAB Review of Toxicology Data Submitted by D-I-1-4, Inc. (DP Barcode No.: D204774; Submission No.: S468617; ID No.: 067727-R 1,4 Sight; Chemical ID No.: 055802).

TO: Leonard Cole/Cynthia Giles-Parker
Registration Division (H7505C)

FROM: Cindy Schaffer, Microbiologist C. Schaffer
Biological Pesticides Section
Science Analysis Branch
Health Effects Division (H7509C)

THROUGH: Roy Sjoblad, Ph.D, Section Head
Biological Pesticides Section
Science Analysis Branch
Health Effects Division (H7509C)

DATA REVIEW RECORD

Product Name:	1,4 Sight	
Trade Name:	1,4-Dimethylnapthalene	
ID No:	067727-R	
Chemical No.:	055802	
MRID No.'s:	430825-10	Acute Oral Toxicity - Rat
	430825-11	Acute Dermal Toxicity - Rabbit
	430825-12	Acute Inhalation Toxicity - Rat
	430825-13	Primary Eye Irritation - Rabbits
	430825-14	Acute Dermal Irritation - Rabbits
	430825-15	Dermal Sensitization - Guinea pig
	430825-16	Mutagenicity - Ames Test
	430825-17	Genotoxicity
	430825-18	Mutagenicity - Mouse Micronucleus Assay

ACTION REQUESTED: SAB has been asked to review the toxicology data in support of the registration of 1,4 Sight, a potato sprout inhibitor. SAB has also been asked to review and comment on waiver requests for the 90-day feeding, immune response and teratogenicity studies.

1 of 30

CONCLUSION: SAB finds the toxicity data acceptable to support the registration of 1,4 Sight. SAB also found the waiver requests for the 90-day feeding, immune response and teratogenicity studies acceptable based on the lack of significant toxicity demonstrated in the studies submitted, insignificant exposure for the proposed uses (see 10/7/94 memorandum from G. Jeffrey Herndon to Cynthia Giles-Parker/James Stone), and natural occurance in food.

SUMMARY OF DATA SUBMITTED:

Acute Oral Toxicity:
The median oral LD_{50} of 1,4-DMN was determined to be 2730 mg/kg of rat body weight.
CLASSIFICATION: ACCEPTABLE-TOX CATEGORY III

Acute Dermal Toxicity (Limit test):
1,4-DMN exhibited a dermal LD_{50} of greater than 2 g/kg rabbit body weight.
CLASSIFICATION: ACCEPTABLE-TOX CATEGORY IV

Acute Inhalation Toxicity:
Rats exposed to a respirable dose of 1,4-DMN displayed an LC_{50} greater than 4.16 mg/L.
CLASSIFICATION: ACCEPTABLE-TOX CATEGORY IV

Primary Eye Irritation:
1,4-DMN produced moderate ocular irritation in all rabbits at 24 hours post dose administration. Irritation dissipated by day 21.
CLASSIFICATION: ACCEPTABLE-TOX CATEGORY II

Acute Dermal Irritation:
1,4-DMN produced a moderate irritation when a single 0.5 ml dose was administered dermally. Dermal irritation dissipated by day 14.
CLASSIFICATION: ACCEPTABLE-TOX CATEGORY IV

Skin Sensitization:
Dermal sensitization was not apparent when 1,4-DMN was applied to guinea pigs when using the modified Bueller method.
CLASSIFICATION: ACCEPTABLE

Mutagenicity Assay (AMES):
Ninety-six point four percent pure 1,4-DMN, in the presence or absence of metabolic activation homogenate, is not a mutagen for any *S. typhimurium* strains tested.
CLASSIFICATION: ACCEPTABLE

Mutagenicity Assay (DNA Synthesis):
The test material did not appear to induce nuclear grain counts at the tested concentration range of 0.25 µg/ml to 10 µg/ml. 1,4-DMN is inactive in the *in vitro* test for unscheduled DNA synthesis in rat liver primary cell culture.
CLASSIFICATION: ACCEPTABLE

011305

Mutagenicity Assay (In-Vivo Micronucleus Assay):
1,4-DMN did not increase the number of micronuclei per 1000 polychromatic erythrocytes in the bone marrow of the CD-1 mouse at doses of 225 mg/kg, 450 mg/kg and 900 mg/kg.
CLASSIFICATION: ACCEPTABLE
Hypersensitivity Incidents:
None reported. The registrant must notify the Agency of any hypersensitivity incidents.

Tox Chemical No.:055802 File Last Updated:_____ Current Date:September 29, 1994

Study; Lab; Study#; Date	Material	MRID #	Results	Tox Category	CORE GRADE
Acute Oral Toxicity-Rat ITT Research Institute #L08456 #6,7,8 Nov. 1993	94.7% 1,4DMN	430825-10	The oral median LD_{50} of 1,4-DMN was determined to be 2730 mg/kg of rat body weight.	III	Acceptable
Acute Dermal Toxicity-Rabbit ITT Research Institute L08456 #4 Oct. 1993	94.7% 1,4-DMN	430825-11	The dermal LD_{50} of 1,4-DMN was determined to be greater than 2 g/kg rabbit body weight.	IV	Acceptable
Acute Inhalation Toxicity-Rat ITT Research Institute L08456L001 Oct. 1993	94.7% 1,4-DMN	430825-12	The LD_{50} of 1,4-DMN, in a respirable dose, is greater than 4.16 mg/L in rats.	IV	Acceptable
Primary Eye Irritation-Rabbit ITT Research Institute L08456 #1 Oct. 1993	94.7% 1,4-DMN	430825-13	Moderate ocular irritation was observed in all rabbits at 24 hrs. post dose admin-istration. Irritation dissipated by day 21.	II	Acceptable
Acute Dermal Irritation-Rabbit ITT Research Institute Oct. 1993	94.7% 1,4-DMN	430825-14	1,4-DMN produced a moderate irritation when a single 0.5 ml dose was applied dermally. Dermal irritation dissipated by day 14.	IV	Acceptable

011305

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Tox Chemical No.:055802 File Last Updated:_____ Current Date:September 29, 1994

Study; Lab; Study#; Date	Material	MRID #	Results	Tox Category	CORE GRADE
Skin Sensitization-Guinea Pig (mod. Bueller) ITT Research Institute #L08456 #5 Oct. 1993	94.7% 1,4-DMN	430825-15	Dermal sensitization was not apparent when 1,4-DMN was applied to guinea pigs.	N/A	Acceptable
Mutagenicity Assay (Ames) Hazelton Washington 15683-0-401 21 Sept. 1993	96.4% 1,4-DMN	430825-16	96.4% pure 1,4-DMN, in the presence or absence of metabolic activation homogenate, is not a mutagen for any *S. typhimurium* strain tested.	N/A	Acceptable
Genotoxicity Test (DNA Synthesis) Hazelton Washington 15683-0-447 28 Sept. 1993	96.4% 1,4-DMN	430825-17	The test material did not appear to induce nuclear grain counts at the tested concentration range of 0.25 μg/ml to 10 μg/ml. 1,4-DMN is inactive in the *in vitro* test for unscheduled DNA synthesis in rat liver primary cell culture.	N/A	Acceptable
Mutagenicity Assay (*In-vivo* Micronucleus Test) Hazelton Washington 15683-0-455 24 Aug. 1993	96.4% 1,4-DMN	430825-18	1,4-DMN did not increase the number of micronuclei per 1000 polychromatic erythrocytes in the bone marrow of CD-1 mice at doses of 225 mg/kg, 450 mg/kg and 900 mg/kg.	N/A	Acceptable

Tox Chemical No.:055802 File Last Updated:_____ Current Date:September 29, 1994

Study; Lab; Study#; Date	Material	MRID #	Results	Tox Category	CORE GRADE
90 - Day Feeding Study	1,4-DMN	N/A	Waived for the current use pattern based on a lack of toxicity in the acute battery of tests.	N/A	N/A
Immune Response	1,4-DMN	N/A	Waived for the current use pattern based on a lack of toxicity in the acute battery of tests.	N/A	N/A
Teratogenicity Studies	1,4-DMN	N/A	Waived for the current use pattern based on a lack of toxicity in the acute battery of tests.	N/A	N/A

011305

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Ph.D., Biologist, SAB/HED

STUDY TYPE:	Acute Oral Toxicity - Rat (152B-10)
MRID NO:	430825-10
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	L08456
STUDY NO.'S:	6, 7, 8
SPONSOR:	D-I-1-4, Inc., Boise, ID
TESTING FACILITY:	ITT Research Institute, Chicago, IL
TITLE OF REPORT:	Acute Oral Toxicity Study of 1,4-Dimethylnaphthalene (1,4-DMN) in Rats.
AUTHOR(S):	William D. Johnson, Ph.D., D.A.B.T.
STUDY COMPLETED:	November 1993
CONCLUSION:	The median oral LD_{50} of 1,4-Dimethylnaphthalene was determined to be 2730 mg/kg of rat body weight.
CLASSIFICATION:	ACCEPTABLE - TOX CATEGORY III

I. STUDY DESIGN

Test Material: The biochemical pest control agent is 1,4-Dimethylnaphthalene (1,4-DMN), lot no.: H5510. The nominal concentration was determined to be 94.7% 1,4-DMN by the sponsor. Stock solutions of the test material were prepared in corn oil at either a 25% w/v (study 6), 30% w/v (study 7) or 50% w/v (study 8) concentration: 750, 1000 or 2500 mg/kg respectively; and diluted to the appropriate dose concentration (see below).

TABLE 1	DOSE LEVEL OF 1,4-DMN MG/KG (5 RATS/SEX)		
STUDY 6	750	1000	2500
STUDY 7	1300	1700	2100
STUDY 8	2000	2300	2500

Test Animals: Forty five male and forty five female Sprague Dawley (Crl:CD^{R}BR) rats were obtained from Charles River Laboratories, Portage, MI. The male rats weighed between 132g and 276g and female weights ranged from 129g to 195g at the beginning of all the studies.

Methods: Five male and five female rats were each treated orally, by gavage, (10ml/kg body weight) with the

test substance at the concentrations listed in table 1. The rats were randomly weighed before initial dosing, and weekly thereafter. Animals were observed for clinical signs and mortality at 1, 3 and 5 hours post dosing and twice daily for 14 days. All rats were examined by necropsy for any macroscopic abnormalities at the end of the study.

II. RESULTS

A. Body Weights: No abnormalities were noted in body weights or weight gain throughout the study.

B. Clinical Observations*:

Clinical Sign	Dose (mg/kg)	Incidence
Discoloration around mouth:	750	2 ♂, 1 ♀
	1000	2 ♂, 2 ♀
	1300	4 ♂, 4 ♀
	1700	4 ♂, 2 ♀
	2000	1 ♀
	2100	5 ♂, 4 ♀
	2300	3 ♂
	2500	7 ♂, 5 ♀
Redness around nose fur:	750	2 ♂
	1000	2 ♂, 4 ♀
	1300	2 ♂, 2 ♀
	1700	4 ♂, 5 ♀
	2000	3 ♂, 4 ♀
	2100	3 ♂, 4 ♀
	2300	2 ♂, 2 ♀
	2500	9 ♂, 7 ♀
Salivation:	750	1 ♂
	1000	1 ♀
	1300	4 ♂, 3 ♀
	1700	4 ♂, 2 ♀
	2000	2 ♂, 2 ♀
	2100	5 ♂, 3 ♀
	2300	4 ♂
	2500	5 ♂, 6 ♀
Hunched posture:	1000	2 ♂, 4 ♀
	1300	5 ♂, 5 ♀
	1700	5 ♂, 5 ♀
	2000	1 ♀
	2100	5 ♂, 5 ♀
	2300	1 ♀
	2500	5 ♂, 4 ♀

Lacrimation:	1000	2 ♀
	1300	3 ♂, 4 ♀
	1700	5 ♂, 5 ♀
	2000	2 ♂, 3 ♀
	2100	4 ♂, 3 ♀
	2300	2 ♂, 4 ♀
	2500	6 ♂, 8 ♀
Clear nasal discharge:	1000	1 ♀
	1300	1 ♀
	2000	1 ♂, 1 ♀
	2100	1 ♂
	2500	3 ♂, 5 ♀
Wet inguinal fur:	1000	1 ♀
	2000	1 ♀
	2500	1 ♂, 2 ♀
Chromodacryorrhea:	1300	1 ♀
Diarrhea:	2100	1 ♀
Discolored inguinal fur:	1700	2 ♂, 1 ♀
	2000	1 ♀
	2100	1 ♀
	2300	1 ♀
	2500	2 ♀
Discolored paws:	2500	4 ♂, 3 ♀
Redness around eyes:	1300	1 ♀
	1700	1 ♀
	2100	1 ♀
	2500	1 ♀
Red nasal discharge:	1300	1 ♂
	1700	1 ♀
	2500	1 ♂
Hair loss:	2000	1 ♀
	2500	1 ♂, 1 ♀
Irritability:	2500	1 ♀
Hypoactivity:	1300	3 ♂, 3 ♀
	1700	5 ♂
	2000	4 ♂, 5 ♀
	2100	3 ♂, 1 ♀
	2300	5 ♂, 5 ♀
	2500	7 ♂, 7 ♀
Ataxia:	2500	2 ♂, 3 ♀

Cold to touch:	2300	1 ♂.
Coma:	2500	2 ♂, 1 ♀
Death:	1700	1 ♀
	2000	1 ♂
	2100	1 ♀
	2300	1 ♂
	2500	7 ♂, 3 ♀

*NOTE: The actual time period for each clinical sign was not reported; except for death. All animals died within the first three days of testing.

C. Necropsy observations:

Observation	*Dose (mg/kg)*	*Incidence*
Red Brain:	2500	5 ♂, 6 ♀
Red Lungs:	2500	3 ♂, 2 ♀
Pale Kidneys:	2300	1 ♀
	2500	6 ♂, 2 ♀
Tan/mottled Kidneys:	1700	1 ♀
	2500	1 ♂
Enlarged Adrenal Glands:	2500	1 ♀
Dark Adrenal Glands:	1700	1 ♀
Red Small Intestines:	2100	1 ♀
Small Testes:	1300	1 ♂
Pale Liver	1700	1 ♀
	2500	5 ♂
Tan/mottled Liver:	1700	1 ♀
Pale Spleen:	2300	1 ♂
	2500	2 ♂
Mottled/dark Thymus:	2500	3 ♂, 3 ♀
Dark fluid in Stomach:	2500	2 ♂

Thin Stomach Mucosa:	2500	1 ♀
Compacted Cecum:	2500	2 ♂
Distended Urinary Bladder:	2500	4 ♂

III. SAB DISCUSSION: All mortality occurred within the first three days; and clinical signs of the remaining animals diminished within the first week post dosing. The gross necropsy findings reportedly represent autolytic changes, not those related to the test substance. Overall, the median oral LD_{50} was determined to be 2730 mg/kg rat body weight.

011305

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Ph.D., Biologist, SAB/HED

STUDY TYPE:	Acute Dermal Toxicity [limit test]-Rabbit (152B-11)
MRID NO:	430825-11
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	L08456/Study #4
SPONSOR:	D-I-1-4, Inc., Boise, ID
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Acute Dermal Toxicity Study of 1,4-Dimethylnaphthalene (1,4-DMN) in Rabbits (Limit Test).
AUTHORS(S):	William D. Johnson, Ph.D., D.A.B.T.
STUDY COMPLETED:	October 1993
CONCLUSION:	The dermal LD_{50} of 1,4-DMN was determined to be greater than 2 g/kg rabbit body weight.
CLASSIFICATION:	ACCEPTABLE - TOX CATEGORY IV

I. STUDY DESIGN

Test Material: The biochemical pest control agent (BPCA) is 1,4-Dimethylnaphthalene (1,4-DMN), batch number H5510. The nominal concentration was determined to be 94.7% 1,4-DMN (MRID #430825-05). The dose, 2 g/kg, was determined by volume (density of 1.016 g/ml per sponsor) but administered as received by weight.

Test Animals: Five male and five female New Zealand White rabbits, approximately 3 months old, were obtained from Kuiper Rabbit Ranch, Gary, IN. The rabbits weighed between 2.52 kg and 2.89 kg at the beginning of the study.

Methods: Twenty-four hours prior to testing, approximately 10% of the trunk fur was clipped. The BPCA was administered, undiluted as received, over the prepared skin followed by surgical dressing, plastic film, a lint-free cloth and an elastic adhesive bandage. All wrappings were removed 24 hours post dosing, the skin was wiped clean using a 0.9% saline moistened gauze pad and towel dried. The animals were observed for signs of toxicity frequently day 1 and daily thereafter. Body weights were recorded on day 1, day 8 and day 15. At the end of the study, all animals were sacrificed by anesthetic overdose and subjected to gross necropsy.

II. RESULTS

A. Body Weights:
One male rabbit failed to gain weight throughout the study and one female displayed a slight loss in body weight from week 1 to week 2.

B. Clinical Observations:
All animals displayed erythema, edema and eschar formation throughout the study and new or repaired skin by the end of the study.

C. Necropsy observations:
Two males and one female exhibited pale kidneys.

One male and one female had eschar formation upon necropsy.***

*** The clinical observations show two males and two females with eschar formation on the last day of the study.

III. SAB DISCUSSION:

Although all animals displayed eschar formation during the study, no mortality or unusual observations were made during necropsy. The LD_{50} of 1,4-DMN was determined to be greater than 2 g/kg rabbit body weight.

011305

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Ph.D., Biologist, SAB/HED

STUDY TYPE:	Acute Inhalation Toxicity Study - Rat
MRID NO:	440825-12
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	L08456L001
SPONSOR:	D-I-1-4, Inc., Biose, ID
TESTING FACILITY:	ITT Research Institute, Chicago, IL
TITLE OF REPORT:	Acute Inhalation Toxicity Study of 1,4-Dimethylnaphthalene (1,4-DMN) in rats.
AUTHOR(S):	Narayanan Rajendran, Ph.D.
STUDY COMPLETED:	October 1993
CONCLUSION:	The LC_{50} of 1,4-DMN, in a respirable dose, is greater than 4.16 mg/L in rats.
CLASSIFICATION:	ACCEPTABLE - TOX CATEGORY IV

I. STUDY DESIGN

Test Material: The biochemical pest control agent is 1,4-Dimethylnaphthalene, batch number H5510. The test material purity was determined to be 94.7% by the sponsor. The test animals received a respirable aerosol dose of 4.16 mg/L.

Test Animals: Five male and five female Sprague-Dawley rats, were obtained from Charles River Laboratories, Kingston, NY. The male rat's weight ranged from 274 gm to 284 gm and the female's weight ranged from 187 gm to 225 gm at the beginning of the study.

Methods: The treated animals received a dose of 4.16mg/L in a respirable aerosol. A continuous supply of fresh test atmosphere was generated by a 'Laskin type' stainless-steel aspirator to produce a whole body exposure. To determine the test material concentration, filter samples were collected once each hour. To determine the amount of test substance, the samples were analyzed by a gas chromatograph. Particle size distribution was analyzed using an Anderson cascade impactor twice during exposure. A physical examination of the test animals was performed each hour during exposure, each half-hour after exposure (to 1.5 hours), and once daily, beginning the day after exposure, for 14 days. Body weights were recorded for both groups prior to exposure, day 7, day 14. Gross necropsy was performed on all animals at the end of the study.

II. RESULTS

A. Exposure Chamber:

The median aerodynamic diameter of particulates was 2.82 μm with a standard deviation of 1.67.

The air flow was reported to be 110 L/minute.

The $\%O_2$ was determined to be 21%.

The relative humidity was 30%.

The chamber temperature was 25.9°C.

B. Clinical Observations:

One female rat was found dead day 1.

All animals experienced discoloration around the nose and wet inguinal fur during the day of dosing and the majority of animals diplayed these symptoms on day 1 post dosing.

Discoloration around the mouth was observed in two males and 5 females the day of dosing. This continued in all females and one male through day 1 post dosing.

Ptosis was observed in three females the day of dosing and one male had ptosis on days 5 and 6.

Dyspnea and hypoactivity were noted in two females the day of dosing; and in four females and one male on day 1.

Two females demonstrated nasal discharge and one male and one female had a discharge from the eyes day 1. One male and one female exhibited nasal discharge and eye discharge on day 2.

One female was found prostrate the day of dosing and two females were found prone on day 1.

C. Animal Body Weights:

No abnormalities were noted in body weights or body weight gain during the study.

D. Necropsy Observations:

No abnormalities were noted upon necropsy.

III. SAB DISCUSSION:

The LC_{50} of 1,4-DMN, in a respirable dose, is greater than 4.16 mg/L in rats.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Ph.D., Biologist, SAB/HED

STUDY TYPE:	Primary Eye Irritation-Rabbit (152B-13)
MRID NO:	430825-13
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	L08456
SPONSOR:	D-I-1-4, Inc., Boise, ID
TESTING FACILITY:	ITT Research Institute, Chicago, IL
TITLE OF REPORT:	Primary Eye Irritancy Study of 1,4-Dimethylnaphthalene (1,4-DMN) in Rabbits.
AUTHOR(S):	William D. Johnson, Ph.D., D.A.B.T.
STUDY COMPLETED:	October 1993
CONCLUSION:	Moderate ocular irritation was observed in all rabbits at 24 hours post dose administration. Irritation dissipated by day 21.
CLASSIFICATION:	ACCEPTABLE - TOX CATEGORY II

I. STUDY DESIGN

Test Material: The biochemical pest control agent (BPCA) is 1,4-Dimethylnaphthalene (1,4-DMN). The purity was determined to be 94.7 by the sponsor. The BPCA was administered undiluted.

Test Animals: Three male and three female New Zealand White rabbits were obtained from Kuiper Rabbit Ranch, Gary, IN. The rabbits were approximately 3 months old and weighed between 2.3 kg and 2.7 kg at the beginning of the study.

Methods: Twenty-four hours prior to MPCA administration, a preliminary ocular screen using a 2% fluorescein solution to evaluate corneal lesions was performed.

A single dose of 0.1 ml of the BPCA was administered into the conjunctival sac of the right eye in each animal. The eye was gently held together for 2 seconds to prevent a loss of material. The left eye served as the control for each animal. The Draize Method was used to score ocular lesions at 1 hour, and 1, 2, 3, 7, 14 and 21 days post dosing. Body weights were recorded on day 1 prior to test material administration. All animals were observed daily for morbidity and mortality.

II. RESULTS

A. Clinical Observations:

Two rabbits vocalized immediately upon test substance instillation.

Three rabbits exhibited circumocular alopecia "during the study".

B. Eye Irritation Scoring:

OBSERVATION	TIME	# RABBITS*	AVE. DRAIZE SCORE
Erythema:	1 hr	6/6	2.0 (moderate)
	1 day	6/6	2.0 (moderate)
	2 days	6/6	1.5 (mild to moderate)
	3 days	4/6	1.3 (mild)
	7 days	4/6	1.3 (mild)
	14 days	1/6	1.0 (mild)
Chemosis:	1 hr	6/6	1.8 (moderate)
	1 day	6/6	2.0 (moderate)
	2 days	6/6	2.0 (moderate)
	3 days	6/6	2.0 (moderate)
	7 days	6/6	1.3 (mild)
	14 days	2/6	1.5 (mild to moderate)
Discharge:	1 day	1/6	2.0 (moderate)
	2 days	2/6	1.5 (mild to moderate)

III. SAB DISCUSSION: Moderate ocular irritation was observed in all rabbits at 24 hours post dose administration. Irritation dissipated by day 21.

011305

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Biologist, SAB/HED

STUDY TYPE:	Primary Dermal Irritation-Rabbit (152-14)
MRID NO:	430825-14
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	L08456
SPONSOR:	D-I-1-4, Inc., Boise, ID
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Acute Dermal Irritancy/Corrosivity of 1,4-Dimethylnaphthalene (1,4-DMN) in Rabbits.
AUTHOR(S):	William D. Johnson, Ph.D., D.A.B.T.
STUDY COMPLETED:	October 1993
CONCLUSION:	Overall, 1,4-DMN produced a moderate irritation in all rabbits averaged over a 72 hour period, when a single 0.5 ml dose was administered dermally for 4 hours. Slight erythema persisted in 3/6 rabbits for 7 days. Dermal irritation dissipated by day 14.
CLASSIFICATION:	ACCEPTABLE - TOX CATEGORY IV

I. STUDY DESIGN

Test Material: The biochemical pest control agent (BPCA is 1,4-Dimethylnaphthalene, batch number H5510. The purity was determined to be 94.7%.

Test Animals: Three male and three female New Zealand White rabbits were obtained from Kuiper Rabbit Ranch, Gary, IN. Body weights ranged from 2.5 to 3.0 kg.

Methods: Approximately 24 hours prior to testing, no less than 240 cm^2 of rabbits' back fur was clipped. The BPCA (0.5 ml) was administered over the prepared skin followed by gauze, Dermiform tape and an elastic wrap used to secure the patch. Approximately 4 hours following application, the gauze patch removed and the site was wiped with 0.9% saline and gauze to remove residual test material. The animals were observed for dermal irritation and corrosivity at 1/2 to 1 hour, 1, 2, 3, 7, and 14 days post dosing. The animals were evaluated for dermal irritation using the Draize method. Body weights were recorded on prior to testing.

011305

II. RESULTS

A. Dermal Irritation Scoring:

Erythema: All males and females displayed very slight to slight redness through day 3. Erythema was noted in all females on day 7.

Edema: All males and females had well-defined to moderate swelling within the first 24 hours post dosing. One male and one female showed signs of slight edema on day 3.

III. SAB DISCUSSION:

The primary irritation index for 1,4-DMN over a 72 hour period is 2.7, equivalent to a moderate irritant. Slight irritation was noted 72 hours post dose administration.

011305

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Ph.D., Biologist SAB/HED

STUDY TYPE:	Skin Sensitization Study-Guinea Pig (152B-15)
MRID NO:	430825-15
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	L08456
SPONSOR:	D-I-1-4, Inc., Boise, ID
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Dermal Sensitivity of 1,4-Dimethylnaphthalene (1,4-DMN) in Guinea Pigs Using the Modified Buehler Method.
AUTHOR(S):	William D. Johnson, Ph.D., D.A.B.T.
STUDY COMPLETED:	October 1993
CONCLUSION:	Dermal sensitization was not apparent when 1,4-DMN was applied to Guinea Pigs.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: The Biochemical pest control agent is 1,4-Dimethylnaphthalene. The purity was determined to be 94.7%. A preliminary study was performed to determine the highest non-irritant concentration and threshold irritation concentration to be used for the dermal induction and challenge applications. Concentrations of 100%, 75%, 50% and 25% v/v test material in white mineral oil was applied to exposed test sites. Based on the results of this study, a concentration of 75% v/v of the test substance in white mineral oil was used for the epidermal induction, and the challenge application consisted of a 50% v/v 1,4-DMN solution in white mineral oil.

Test Animals: Twenty male albino Hartley guinea pigs were obtained from Sasco, Inc., Omaha, NE. The guinea pigs weighed between 375 and 453 grams at dosing.

Methods: The guinea pig maximization test consists of two stages. The induction phase, which consists of a three week series of topical dermal applications (0.3 ml @ 75% v/v dose in a hilltop chamber), on the upper left quadrant of ten guinea pigs backs, then wrapped with an elastic adhesive bandage. followed 14 days later by a challenge dose, of the test material (0.3 ml @ 50% v/v dose). Six hours post application, the bandages were removed. Ten guinea pigs in the control group received similar treatment with the omission of the test material

during the induction phase. The sham controls received the same challenge dose of the test material as the test group (0.3 ml of 50% v/v test material in white mineral oil). The skin reactions at the challenge site were assessed twenty four and forty eight hours after the dressings were removed and graded according to the following scale:

REACTION	VALUE
No reaction	0
Slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema to slight eschar formation	4

Each guinea pig was weighed once during the first week and weekly thereafter. The animals were observed daily for signs of toxicity and mortality.

II. RESULTS

A. Skin Reactions in Test Animals after induction:
Nine out of ten treated guinea pigs experienced very slight erythema within 24 hours; while seven guinea pigs showed signs of very slight erythema at 42 hours post induction.

B. Skin Reactions in Test Animals after challenge application:
Treated animals: Seven guinea pigs displayed signs of very slight erythema and three animals had well defined erythema within the first 24 hours post dosing. By 48 hours post dosing, very slight erythema was noted in 5/10 guinea pigs.
Sham control animals: Eight guinea pigs displayed signs of very slight erythema and two animals had well defined redness within the first 24 hours post dosing. By 48 hours post dosing, very slight erythema was noted in 5/10 guinea pigs.

C. Body Weights:
No abnormalities in body weight gain were noted.

E. Clinical signs/Mortality:
No signs of toxicity or mortality were noted during the study.

II. SAB DISCUSSION:
Since slight positive reactions were noted equally after the challenge dose in both the sham control and treated animals; 1,4-DMN is not considered a dermal sensitizer.

011305

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Ph.D., Biologist, SAB/HED

STUDY TYPE:	Mutagenicity Assay (Ames Assay) (84-2)
MRID NO:	430825-16
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	15683-0-401
SPONSOR:	D-I-1-4, Inc. Boise, ID
TESTING FACILITY:	Hazelton Washington, Inc., Vienna, VA
TITLE OF REPORT:	Mutagenicity Test on 1,4-Dimethylnaphthalene in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test).
AUTHORS(S):	Timothy E. Lawlor, M.A.
STUDY COMPLETED:	21 September, 1993
CONCLUSION:	Ninety-six point four percent pure 1,4-DMN, in the presence or absence of metabolic activation homogenate, is not a mutagen for any *S. typhimurium* strains tested.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: 1,4-Dimethylnaphthalene (1,4-DMN), Batch H5510 (96.4% pure).

Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 amd TA1538.

S-9 homogenate; prepared from livers of Sprague-Dawley rats induced with Arochlor.

The following were utilized as positive controls: The activated tester strains TA98, TA100, TA1535, TA1537 and TA1538 used 2-aminoanthracene (2.5 µg); The non-activated tester strains TA98 and TA1538 utilized 2-nitrofluorene (1.0µg); Strains TA100 and TA1535 had sodium azide (2.0 µg); and TA1537 used ICR-191 (2.0 µg).

Methods: A preliminary study was performed to determine the range of doses to be tested. The results of the study indicate that in the presence of S9 mix, the doses should range from 10 µg to 1000 µg per plate; and in the absence of S9 mix, the dose range will be 1 µg to 250 µg of the test material per plate.

The mutagenicity of 1,4-DMN to five strains of *S. typhimurium* was evaluated using the test substance at 10.0µg, 50.0µg, 100µg, 250µg, 500µg and 1000µg

per assay plate, when activated with rat liver microsomes; and at a concentration of 1.0µg, 5.0µg, 10µg, 25µg, 50µg and 250µg per assay plate, without the S-9 rat liver microsomes. The vehicle control was DMSO. Three individual studies were performed to evaluate the mutagenicity of the test material; with two of these assays specifically studying tester strain TA1538. Three plates per treatment were used for enumeration of revertant colonies.

II. RESULTS: The effect of 1,4-Dimethylnaphthalene to induce reverse mutations in *S. typhimurium* test strains (average of all three studies):

Compound / Vehicle	Dose level (ug/plate) 50µl	Mean number of revertant colonies formed with strains: TA98 +	TA98 −	TA100 +	TA100 −	TA1535 +	TA1535 −	TA1537 +	TA1537 −	TA1538 +	TA1538 − *
		33	12	133	97	14	11	10	4	16	14
1,4-Dimethyl-naphthalene	1.0µg	-	12	-	89	-	8	-	7	-	15
	5.0µg	-	14	-	114	-	10	-	5	-	12
	10.0µg	26	13	173	107	13	12	6	5	15	11
	25.0µg	-	11	-	93	-	4	-	3	-	11
	50.0µg	24	11	184	90	12	9	9	5	17	11
	100.0µg	26	-	190	-	11	-	9	-	20	-
	250.0µg	30	5	142	54	11	5	5	4	27	1
	500.0µg	31	-	93	-	13	-	2	-	0	-
	1000.0µg	9	-	13	-	3	-	0	-	0	-
Positive controls											
2-nitrofluorene		-	138	-	-	-	-	-	-	-	241
2-aminoanthracine		677	-	452	-	323	-	234	-	989	-
Sodium azide		-	-	-	776	-	129	-	-	-	-
ICR-191		-	-	-	-	-	-	-	96	-	-

* (+) = activated; (-) = non-activated ' = Concentration in µl/plate

III. SAB DISCUSSION:
The test material did not appear to induce mutagenicity or dose related bacterial toxicity in any *S. typhimurium* test strain during this study.

011305

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Ph.D., Biologist, SAB/HED SR

STUDY TYPE:	Mutagenicity Assay (In-vivo Micronucleus Assay) (84-2)
MRID NO:	430825-18
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	15683-0-455
SPONSOR:	D-I-1-4, Inc. Boise, ID
TESTING FACILITY:	Hazelton Washington, Inc., Vienna, VA
TITLE OF REPORT:	Mutagenicity Test on 1,4-Dimethylnaphthalene *In Vivo* Mouse Micronucleus Assay.
AUTHORS(S):	Hemalatha Murli, Ph.D.
STUDY COMPLETED:	24 August, 1993
CONCLUSION:	Although the test material did not increase the number of micronuclei per 1000 polychromatic erythrocytes in the bone marow of the CD-1 mouse at doses of 225 mg/kg, 450 mg/kg and 900 mg/kg, the PCE:NCE ratio decreases with an increased dose of 1,4-DMN. This may indicate a suppressive effect on the bone marrow.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: 1,4-Dimethylnaphthalene (1,4-DMN), Batch H5510 (96.4% pure) solubilized in corn oil.

Positive control: Cyclophosphamide solubilized in deionized water.

Vehicle control: Corn oil.

Test Animals: Sixty male and sixty female CD-1 mice were obtained from Charles River Laboratories, Raleigh, NC..

Methods: *Dose Selection:* Two preliminary studies were performed to determine the range of doses to be tested. In the first study, three animals of each sex received either 500mg/kg, 1625 mg/kg, 2750 mg/kg, 3875 mg/kg, or 5000 mg/kg of 1,4-DMN by oral gavage. All but one animal died when given a dose of 1625 mg/kg or higher. Since these results were not sufficient to select a dose range, an additional study was conducted testing dose levels of 900 and 1300 mg/kg. One out of six animals died when treated with 900 mg/kg while 2/6 mice died when dosed with 1300 mg/kg of the test material.

The results of this study indicate that the maximum tolerated dose (MTD) was 900 mg/kg 1,4-DMN. The doses selected for this assay were 225, 450 and 900 mg/kg body weight.

The test substance, diluted in corn oil, was administered by oral gavage, to five mice of each sex per treatment group at a volume of 10 ml/kg. An additional five males and five females were orally dosed with either 80 mg/kg of the positive control; or 10 ml/kg of the vehicle control. The treated animals were terminated 24, 48 or 72 hours post dosing; while the control mice were euthanized with CO_2 within 24 hours after dose administration. The bone marrow was aspirated from the tibia of the mice, and mixed with 0.1 ml fetal bovine serum (FBS). The cells were placed on a slide, air dried, fixed in methanol, and stained with May-Grunwald solution and Giemsa. A coverslip was mounted on each slide with Depex mounting medium. Each slide was scored for micronuclei as well as the polychromatic (PCE) to normochromatic (NCE) cell ratio. One thousand PCE's were counted. The frequency of micronucleated cells was based on the percent of micronucleated cells per total PCE's present in the optic field. The normal range of micronuclei in CD-1 mice is 0.0% to 0.4%. The frequency of PCE's versus NCE's was determined by grading the number of PCE's and NCE's observed in the optic fields while counting the first 1000 erythrocytes.

II. RESULTS: The effect of 1,4-Dimethylnaphthalene to induce an increase the frequency of micronuclei per 1000 polychromatic erythrocytes in mouse bone marrow; average of five animals per sex:

Compound	Dose (mg/kg)	%. mean PCE's (1000) ♂	%. mean PCE's (1000) ♀	Ratio PCE:NCE mean ♂	Ratio PCE:NCE mean ♀
Vehicle control	10 ml/kg	0.02	0.04	0.77	0.92
Positive control	80	0.82	1.16	0.96	0.97
1,4-DMN @ 24 hrs	225	0.12	0.02	0.71	1.00
	450	0.04	0.04	0.84	0.92
	900	0.14	0.00	0.87	0.94
1,4-DMN @ 48 hrs	225	0.06	0.20	0.54	0.48
	450	0.06	0.06	0.54	0.37
	900	0.10	0.02	0.56	0.44

1,4-DMN @ 72 hrs	225	0.10	0.02	0.58	0.32
	450	0.06	0.00	0.54	0.45
	900	0.04	0.02	0.48	0.38

III. SAB DISCUSSION:

Although the test material did not increase the number of micronuclei per 1000 polychromatic erythrocytes in the bone marow of the CD-1 mouse at doses of 225 mg/kg, 450 mg/kg and 900 mg/kg, the PCE:NCE ratio decreases with an increased dose of 1,4-DMN. This may indicate a suppressive effect on the bone marrow.

011305

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: Sheryl Reilly, Ph.D., Biologist, SAB/HED

STUDY TYPE:	Genotoxicity Test (DNA synthesis) [84-2(3)(i)(c)]
MRID NO:	430825-17
TEST MATERIAL:	1,4-Dimethylnaphthalene
SYNONYMS:	1,4-DMN
PROJECT NO:	15683-0-447
SPONSOR:	D-I-1-4, Inc. Boise, ID
TESTING FACILITY:	Hazelton Washington, Inc., Vienna, VA
TITLE OF REPORT:	Genotoxicity Test on 1,4-Dimethylnaphthalene In the Assay for Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures.
AUTHORS(S):	Andrea L. Ham, B.S.
STUDY COMPLETED:	28 September, 1993
CONCLUSION:	The test material did not appear to induce nuclear grain counts at the tested concentration range of 0.250 µg/ml to 10 µg/ml. 1,4-Dimethylnaphthalene is inactive in the *in vitro* test for unscheduled DNA synthesis in rat liver primary cell culture.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: 1,4-Dimethylnaphthalene (1,4-DMN), Batch H5510 (96.4% pure).

Indicator cells: Hepatocytes, obtained from a single adult male Fischer 344 rat, cultured in monolayers in Williams' Medium E supplemented with 2 mM L-glutamine, 100 µg/ml streptomycin sulfate, 150 µg/ml gentamycin, and with [WME+] or without 10% fetal bovine serum (FBS) [WMEI], depending upon the phase of growth. The latter phase was cultured in WMEI. The treatment phase of the study contained 10 µCi/ml ^{3}HTdr (46 Ci/mMole) [WME-treat].

Solvent control: Dimethylsulfoxide (DMSO).

Positive control: 2-Acetylaminofluorene (2-AAF).

Stock solution: Fresh preparations of the test material in DMSO were prepared at the highest desired concentration and serially diluted with DMSO; then diluted 1:100 into WME-treat.

Methods:

Indicator cells were prepared by perfusion of rat livers *in situ* with Hanks balanced salt solution (HBSS), and subsequently with collagenase/HBSS. The hepatocytes were obtained by a mechanical extraction. After shaking the plates, the cellular debri was allowed to settle and the supernatent was spun down in a centrifuge and reconstituted with WME+. The cells were counted and at least 5 aliquots (of 0.5 x 10^6 viable cells in 3 ml of WME+ per dose level) were placed in 35mm culture dishes. The culture dishes used for unscheduled DNA synthesis (UDS) contained coverslips; the others utilized for attachment efficiency and cytotoxicity evaluation did not.

To establish cell cultures, the plates were initially incubated at 37°C/5% CO_2 for 1.8 hours then washed and refed with WMEI. The media was replaced 2.1 hours later with 2.5 ml WMEI + 10µCi/ml ^{3}HTdr (46Ci/Mmole) + the test material [or positive or negative control] at the the following concentrations: 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1 µg/ml, 0.5 µg/ml, and 0.25 µg/ml. The cultures incubated for an additional 18.6 hours. The assay was terminated by washing the cultures twice with WMEI. Three cultures had an additional wash of WMEI + 1Mm Thymidine. The other two cultures were refed with WMEI, incubated for an additional 20.3 hours and viable cell counts relative to the negative control were obtained through trypan blue exclusion.

An addition of one percent sodium citrate to the coverslips for 10 minutes allowed swelling of the cell nuclei. The cells were then fixed with a 1:3 ratio of acetic acid:ethanol and dried for at least 24 hours. The coverslips were then mounted onto glass slides, coated with an emulsion of Kodak NTB2 and deionized water, then dried. The slides were stored in a light tight box containing dessicant for seven days, developed in D19, fixed and stained using Williams' modified hematoxylin and eosin method.

UDS (or net nuclear grain count) was measured by examining the cells, at 1500X magnification, counting the nuclear grains on at least 50 randomly selected cells from each coverslip, and subtracting the background count of grains in a three nuclear-sized areas adjacent to each nucleus.

II. RESULTS: The effect of 1,4-Dimethylnaphthalene to induce an increase of net nuclear grain (NNG) counts in rat liver primary cell cultures:

Compound	Dose level (µg/ml)	Mean NNG	% Survival @ 20 hrs
Solvent control	1%	-2.68	100.0
Positive control	0.1	7.35	89.4
1,4-DMN	10.0	0.01	58.2
	5.0	-1.38	95.8
	2.5	-2.77	108.9
	1.0	-1.18	106.8
	0.5	-1.37	103.2
	0.25	-1.65	103.0

III. SAB DISCUSSION:
The test material did not appear to induce nuclear grain counts at any tested concentration. 1,4-Dimethylnaphthalene is inactive in the *in vitro* test for unscheduled DNA synthesis in rat liver primary cell culture.

END